

## Ultrastructure of Scutellar Sensilla in *Aphytis melinus* (Hymenoptera: Aphelinidae) and Morphological Variation across Chalcidoidea

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*Abstract.*—Paired, disc-like campaniform sensilla occur on the scutellum of many minute parasitic wasps in the superfamily Chalcidoidea (Hymenoptera). The ultrastructure of the sensilla is examined in *Aphytis melinus* DeBach (Aphelinidae). Each sensillum consists of a bilayered cuticular cap directly covering a tubular body with microtubules extending at a right angle to the cuticle. A large electron-dense mass attached to the tubular body extends laterally beneath the cuticle. Other structures occupying the space between the scutellum and longitudinal flight muscles include the paired mesoscutello-metanotal muscles and a previously undescribed layer of oblong structures lining the cuticle throughout the thorax. Among 23 additional species examined, the sensilla range in diameter from 1.81  $\mu\text{m}$  to 5.79  $\mu\text{m}$ , with no apparent relationship between diameter of the sensilla and size of the scutellum. The function of the sensilla is unknown, but the consistent presence of the sensilla in small chalcidoids and the frequent absence in the largest species suggests a possible association with specialized flight peculiar to small insects obliged to utilize the clap-and-fling flight mechanism.

*Key words.*—campaniform sensilla, morphology, sensory structures

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Chalcidoidea is a diverse superfamily of parasitic Hymenoptera whose species range in length from the smallest known insect (0.11 mm) to relatively large wasps (45 mm), with most specimens averaging 2–4 mm in length (Heraty and Gates 2003). Over 22,000 species of Chalcidoidea are described, making it second to Ichneumonoidea in diversity, but with an estimated 100,000 to 400,000 undescribed species, it may well prove to be the largest superfamily of Hymenoptera (Gibson et al. 1999; Gordh 1975a; Heraty and Gates 2003; Noyes 2000, 2003). Despite over 200 years of taxonomic work, phylogenetic relationships at the family and subfamily levels remain unclear (LaSalle et al. 1997). Difficulties in understanding chalcidoid phylogenetics are due in part to the vast numbers of undescribed species and the poor preservation of many curated specimens (Heraty 2004; LaSalle 1993). Addi-

tionally, with the vast majority of chalcidoids measuring less than 4 mm, there is often a paucity of reliable phylogenetically informative morphological structures.

Sensillar structures have proven to be a rich source of morphological characters, and there have been numerous investigations into the structure and function of sensilla found within Chalcidoidea (Baaren et al. 1996; Barlin and Vinson 1981; Olson and Andow 1993; Schmidt and Smith 1985, 1987). The antenna has been the focal point of the majority of sensillar investigations in chalcidoids due to the high concentration and diversity of antennal sensilla (Barlin and Vinson 1981; Basibuyuk et al. 2000; Olson and Andow 1993; Walther 1983). These studies have focused largely on classifying types of antennal sensilla based on ultrastructural morphology (Amornsak et al. 1998; Baaren et al. 1996; Barlin and Vinson 1981; Basibuyuk and Quicke 1999; Cônsoli et al. 1999; Isidoro et al. 1996; Olson and Andow 1993). Other investiga-

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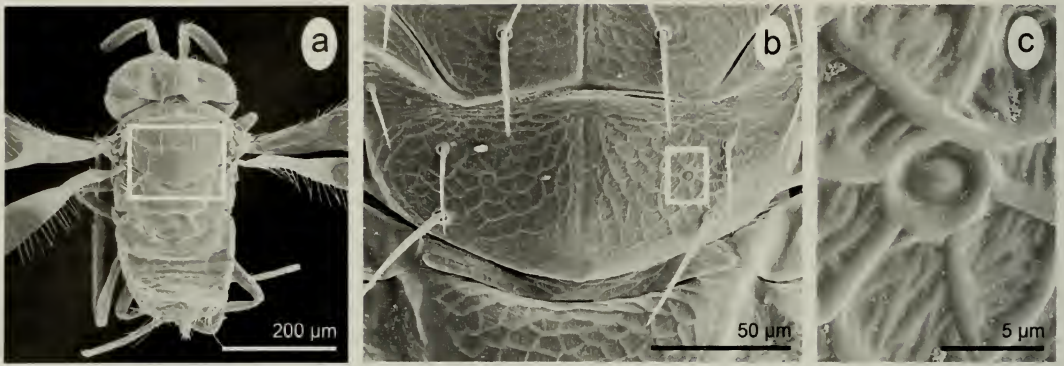


Fig. 1. Location and appearance of scutellar sensilla in *Aphytis melinus*. a: whole specimen, b: scutellum, c: right scutellar sensillum. White boxes in (a) and (b) indicate area magnified in following figure.

tions have examined sensory structures of the ovipositor (Cônsoi et al. 1999; Le Ralec et al. 1996; Le Ralec and Rabasse 1988; Veen and Wijk 1985), male genitalia (Chiappini and Mazzoni 2000) and wings (Schmidt and Smith 1985). The paired sensilla of the scutellum (Fig. 1a–c) have been identified as phylogenetically important (Hayat 1998; Heraty and Polaszek 2000; Kim 2003; Schauff et al. 1996), but there has been no investigation into their ultrastructure, possible function or distribution across Chalcidoidea.

The scutellar sensilla are a feature frequently overlooked in taxonomy and have received only sparse attention in the literature. Domenichini (1969) was one of the first morphologists working with Chalcidoidea to point out the scutellar sensilla, noting their occurrence in several different families and recommending that their function and taxonomic value be studied. Rosen and DeBach (1979) also noted the sensilla in their treatise on *Aphytis* Howard (Aphelinidae), mentioning in each of their species descriptions the location of the scutellar sensilla relative to the anterior and posterior scutellar setae. They observed that, in slide preparations, the sensilla can be mistaken for empty setal sockets due to the thinness of the cuticle over the sensilla. They also noted rare mutations involving the sensilla in which one or both sensilla are replaced by an

extra seta, by a pair of setae, or where there appears to be one, three, or four sensilla in place of the normal pair of sensilla. Both Schauff et al. (1996) and Hayat (1998) incorporated the placement of the sensilla in their keys of *Encarsia* Förster (Aphelinidae). Heraty and Polaszek (2000) used the close placement of the sensilla on the scutellum as a defining characteristic of the *Encarsia strenua* group. Placement of the sensilla on the scutellum was also used by Schauff (1984) as a character in his phylogeny of Mymaridae. Other allusions to the sensilla in the literature are limited to inclusion in illustrations and an occasional mention in species descriptions.

Herein we demonstrate that these scutellar structures are campaniform sensilla, which are circular to oval in shape and innervated by just one sense cell, or neuron, that partially penetrates the thin-domed cuticle (Hicks 1857; Berlese 1909; Snodgrass 1935; McIver 1985). Campaniform sensilla have a mechanoreceptive function targeted at sensing tension or torsion in the associated cuticle (Pringle 1938a; McIver 1985; Zill and Moran 1981).

In chalcidoids, campaniform sensilla have been identified on the antenna (Amornsak et al. 1998; Olson and Andow 1993), ovipositor (Cônsoi et al. 1999; Le Ralec et al. 1996; Le Ralec and Wajnberg 1990), male genitalia (Chiappini and Mazzoni 2000), wing (Schmidt and Smith 1985;



Weis-Fogh 1973), pretarsus (Gladun and Gumovsky 2006) and legs (Schmidt and Smith 1987), but the internal ultrastructure of these sensilla in the superfamily has been examined only in male genitalia of Mymaridae (Chiappini and Mazzoni 2000).

In the current study, *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae: Aphelininae) was chosen to examine the ultrastructure of the scutellar sensilla. *Aphytis melinus* range in size from 0.78 mm to 1.21 mm (Rosen and DeBach 1979) and have been well studied because of their success as biological agents controlling the California Red Scale, *Aonidiella aurantii* (Maskell) (Lenteren 1994). Prior to this study, paired scutellar sensilla were recorded only in the smallest Chalcidoidea such as Aphelinidae (Hayat 1984, 1997; Huang 1994; Heraty and Polaszek 2000; Babcock et al. 2001; Kim 2003; Noyes and Valentine 1989; Schauff et al. 1996), Encyrtidae (Hayat 2003; Noyes 1988; Noyes et al. 1997; Prinsloo 1997), Mymaridae (Schauff 1984), Signiphoridae (Noyes and Valentine 1989) and Trichogrammatidae (Doutt and Viggiani 1968; Noyes and Valentine 1989), with most attention being given to scutellar sensilla in Aphelinidae (Heraty and Polaszek 2000; Kim 2003; Rosen and DeBach 1979).

No previous work has sought to examine the ultrastructure of the scutellar sensilla found in Chalcidoidea. This study seeks to survey variation in external appearance of the scutellar sensilla found in Chalcidoidea, examine the ultrastructure of the sensilla in *A. melinus*, and accurately determine the category of sensilla to which they belong.

## MATERIALS AND METHODS

**Terminology.**—Terms and abbreviations follow Gibson (1997) and Kim (2003) for the structures of the mesonotum, Krogmann and Vilhelmsen (2006) and Vilhelmsen (2000) for muscles and internal morphology, and Harris (1979) for cuticular sculpturing. The paired campaniform sen-

Table 1. Abbreviations used in figures.

112	longitudinal flight muscles
114	mesoscutello-metanotal muscle
2ph	second phragma
ass	anterior scutellum setae
cs	campaniform sensilla
cut	cuticle
edm	electron dense mass
edr	electron dense ring
ela	electron lucent area
epd	epidermal cells
fb	fat body
fl	flange
L1	outer layer
L2	inner layer
m	mitochondria
ml	midline between left and right longitudinal flight muscles
ms	mesoscutum
pss	posterior scutellum setae
scl	scutellum
ssr	scutoscutellar ridge
sss	scutoscutellar suture
tb	tubular body
tsc	transscutal articulation

silla on the scutellum have been termed campaniform sensilla (cs), and terminology specific to structures of the campaniform sensilla follows McIver (1985). Abbreviations are listed in Table 1.

**Specimens.**—*Aphytis melinus* for scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were obtained from a colony reared on *Aspidiotus nerii* Bouché (Diaspididae) at the University of California, Riverside. An additional 30 specimens representing 23 species from ten families of Chalcidoidea, and one specimen of Mymarommatoidea were imaged with SEM. A list of Chalcidoidea used for SEM imaging is given in Table 2; all material is represented by vouchers deposited at the University of California, Riverside Entomology Research Museum (UCRC). The external morphology of the sensilla in Chalcidoidea and outgroups were more broadly surveyed, but this will be treated separately (Romero and Heraty, *in prep.*). Scutellar sensillae have not been documented outside of Chalcidoidea and

Table 2. Sensillum diameters from SEM images. n indicates number of sensilla examined for that species.

Taxon	n	Shape of sensillum	Maximum diameter mean $\pm$ SD (range)	Average area of scutellum
<b>Aphelinidae</b>				
<i>Aphytis melinus</i> DeBach	7	circular	4.94 $\mu\text{m} \pm 0.40$ (3.68–4.94 $\mu\text{m}$ )	14.84 mm
<i>Marietta</i> sp.	4	circular	2.25 $\mu\text{m} \pm 0.27$ (1.93–2.60 $\mu\text{m}$ )	11.18 mm
<i>Ablerus americanus</i> Girault	2	circular	2.86 $\mu\text{m} \pm 0.37$ (2.60–3.12 $\mu\text{m}$ )	6.48 mm
<i>Cales noacki</i> Howard	3	circular	2.99 $\mu\text{m} \pm 0.58$ (2.32–3.39 $\mu\text{m}$ )	6.29 mm
<i>Eretomocerus</i> sp.	2	circular	4.11 $\mu\text{m} \pm 0.17$ (3.99–4.23 $\mu\text{m}$ )	9.58 mm
<i>Eriaphytis</i> sp.	1	circular	5.10 $\mu\text{m}$	26.83 mm
<b>Encyrtidae</b>				
<i>Comperiella bifasciata</i> Howard	4	circular	4.10 $\mu\text{m} \pm 0.38$ (4.14–4.83 $\mu\text{m}$ )	23.71 mm
<i>Microterys nietneri</i> (Motschulsky)	2	circular	5.35 $\mu\text{m} \pm 0.62$ (4.92–5.79 $\mu\text{m}$ )	42.66 mm
<b>Eucharitidae</b>				
<i>Orasema minutissima</i> (Howard)	2	circular	1.87 $\mu\text{m} \pm 0.08$ (1.81–1.93 $\mu\text{m}$ )	27.06 mm
<i>Gollumiella antennata</i> (Gahan)	2	circular	3.47 $\mu\text{m} \pm 0.45$ (2.24–3.79 $\mu\text{m}$ )	45.9 mm
<b>Eulophidae</b>				
<i>Pnigalio</i> sp.	3	subcircular	3.39 $\mu\text{m} \pm 0.82$ (2.82–4.44 $\mu\text{m}$ )	28.36 mm
<i>Pnigalio agraulis</i> (Walker)	2	subcircular	3.93 $\mu\text{m} \pm 0.17$ (3.80–4.05 $\mu\text{m}$ )	34.43 mm
<b>Mymaridae</b>				
<i>Gonatocerus ashmeadi</i> Girault	1	circular	4.87 $\mu\text{m}$	48.76 mm
<b>Pteromalidae</b>				
<i>Philotrypesis</i> sp.	2	subcircular	3.70 $\mu\text{m} \pm 0.11$ (3.78–3.62 $\mu\text{m}$ )	52.76 mm
<i>Asaphes</i> sp.	1	subcircular	3.66 $\mu\text{m}$	30.07 mm
<i>Nasonia vitripennis</i> (Walker)	1	subcircular	4.34 $\mu\text{m}$	NA
<b>Signiphoridae</b>				
<i>Signiphora</i> sp.	2	circular	2.99 $\mu\text{m} \pm 0.20$ (2.85–3.13 $\mu\text{m}$ )	26.54 mm
<b>Tanaostigmatidae</b>				
<i>Tanaostigma</i> sp.	1	circular	3.82 $\mu\text{m}$	56.54 mm
<b>Tetracampidae</b>				
<i>Epiclerus</i> sp.	1	circular	4.86 $\mu\text{m}$	17.80 mm
<b>Torymidae</b>				
<i>Megastigmus transvaalensis</i> (Hussey)	1	circular	5.39 $\mu\text{m}$	101.49 mm
<b>Trichogra mmatidae</b>				
<i>Aphelinoidea</i> sp.	2	circular	5.07 $\mu\text{m} \pm 0.14$ (4.97–5.17 $\mu\text{m}$ )	7.99 mm
<i>Haeckeliana</i> sp.	2	circular	5.05 $\mu\text{m} \pm 0.12$ (4.96–5.13 $\mu\text{m}$ )	10.14 mm
<i>Hayatia</i> sp.	2	circular	3.82 $\mu\text{m} \pm 0.30$ (3.60–4.03 $\mu\text{m}$ )	5.34 mm
Total	50		3.89 $\mu\text{m} \pm 1.01$ (1.81–5.79 $\mu\text{m}$ )	27.91 mm

the majority of outgroup Hymenoptera examined had no trace of sensilla. However, sensilla were found in species from four outgroup families, Ceraphronidae (*Ceraphron* sp.), Diapriidae (*Trichopria* sp.), Mymarommatidae (*Mymaromma anomalum* (Blood & Kryger)) and Scelionidae (*Teleonomus* sp.). These families represent three different superfamilies from the subdivi-

sion Proctotrupomorpha, which includes Chalcidoidea, and Ceraphronidae, representing the more distantly related subdivision Evaniomorpha.

*SEM*.—Specimens selected for SEM were collected in 70% ethanol then dried in hexamethyldisilazane (HMDS) (Heraty and Hawks 1998). Some specimens were gradually rehydrated through a series of



increasingly dilute ethanol baths, rinsed in two baths of deionized water, then digested in 10% KOH for 5–30 min according to the size of the specimen in order to clean the specimen of debris. Specimens were again rinsed in deionized water and dehydrated through a series of increasingly concentrated ethanol baths, then chemically dried in HMDS. Once dry, specimens were either dissected or placed whole onto SEM mounting stubs. Specimens were Au/Pd coated using a Cressington 108 Auto® sputter coater set for 60–90 seconds, then examined and digitally imaged under a XL30 FEG scanning electron microscope at 10 or 15 kV.

*Measurements.*—Scutellar and sensillar measurements were taken in ImageJ 1.38× using the digital SEM images. Width measurements of the scutellum were made across the broadest point of the scutellum, excluding the axillula, and length measurements along the longest medial part of the scutellum including the frenum. Area measurements were made using the free-hand tool in ImageJ. Measurements of the differentiated area of the sensillum were taken along the longest axis and excluding the encircling ring, if present. To determine if there is a correlation between the size of the scutellum and the diameter of the sensilla, the length, width and area of the scutella of 50 specimens were measured (Table 2). A regression line was calculated for each of the three measurements of the scutellum that were graphed, and the coefficient of determination (R-squared) value calculated.

*TEM.*—Live *A. melinus* were decapitated while immersed in Karnovsky's fixative (Karnovsky 1965). After approximately two hours they were placed in sodium cacodylate buffer, dehydrated in ethanol and embedded in Spurr resin (Spurr 1969). Sections approximately 60–70 µm thick were cut using a diamond knife on a Leica Ultracut microtome. Sections were mounted on Electron Microscopy Sciences nickel slot grids coated with formvar/

carbon. Sections were then post stained using the SynapTec GridStick™ system as follows. The uranyl acetate stain was diluted in methanol and the lead citrate stain mixed using 0.3 grams lead citrate, 0.3 grams lead nitrate, 0.3 grams lead acetate and 0.6 grams sodium citrate dissolved in 24.6 ml pre-boiled double distilled deionized water using a sonicator; after sonication 5.4 ml of 1N NaOH solution was added to the lead stain. Grids were initially stained for 5 minutes in uranyl acetate followed by two rinses in 100% methanol, one rinse each in 75%, 50% and 25% methanol, and four rinses in pre-boiled, double-distilled deionized water. The grids were then immediately stained for 10 minutes in the lead stain followed by a 30 second rinse in 0.02 N NaOH and 30 minutes of rinsing with water changed every 5 minutes. Sections were examined with a Philips Tecnai 12 transmission electron microscope and digitally imaged using a model 780 Gatan DualVision 300 camera.

*Slide Mounts.*—*Aphytis melinus* were collected in 70% ethanol and gradually hydrated through a series of increasingly dilute ethanol baths, rinsed in two baths of deionized water and then digested in 10% KOH for 10 minutes. Following digestion, specimens were rinsed in deionized water and dehydrated through a series of increasingly concentrated ethanol baths to 100% ethanol. They were then placed in a well plate with three drops of clove oil and the ethanol allowed to evaporate completely. The antennae, head, wings and body were separated from each specimen and arranged on the slide in 25% Canada Balsam and 75% clove oil (Noyes 2003). As the clove oil evaporated, the Canada Balsam was gradually built up until the structures were covered and four 5 mm coverslips applied.

## RESULTS

In most Apocrita, the mesonotum is divided by the transscutal articulation

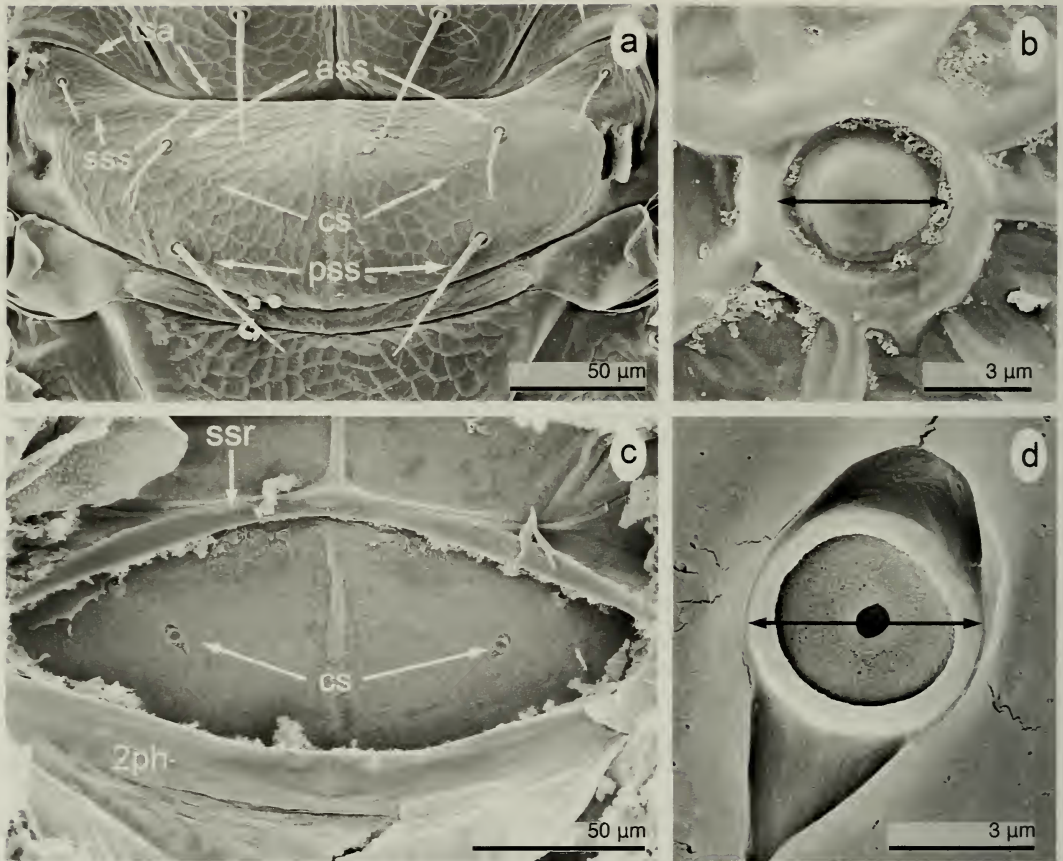


Fig. 2. Structure of scutellum and scutellar sensilla in *Aphytis melinus*. a: scutellum, b: scutellar sensillum, c: underside of scutellum with tissue removed and both sensilla visible, d: underside of sensillum with tissue removed. Black arrows =  $4.84\mu\text{m}$ , indicate equal distance in both (b) and (d).

(Fig. 2a: tsa) into an anterior mesoscutum and a posterior scutellar-axillar complex (Gibson 1997). The medially located scutellum is separated from the anterolateral axilla by the scutoscuteal suture (Fig. 2a: sss). With the exception of Signiphoridae, in which the scutellum is reduced to a transverse band, all Chalcidoidea possess a scutellum that is a prominent plate of variable size and shape. The scutellum can be roughly circular, oval, shield or teardrop shaped and can also vary in topography. For example, some Encyrtidae have a rounded scutellum with sharply rising sides that form a dorsal hump, while some Mymaridae have a flat planar scutellum. Many chalcidoids (i.e. Eucharitidae, Mymaridae, Pteromalidae, Tetracam-

pidae and Torymidae) have a transverse sulcus or change in sculpture differentiating a posterior region of the scutellum termed the frenum. In many taxa, lateral axillular grooves separate the axillula from the main portion of the scutellum, but this is often more apparent in lateral view. The scutellum of many smaller Chalcidoidea often has two pairs of prominent setae: the anterior scutellar setae (Fig. 2a: ass) and the posterior scutellar setae (Fig. 2a: pss). When present, these setae are used as reference points for the campaniform sensilla on the scutellum.

*Structure of scutellum and sensilla in Aphytis.*—*A. melinus* has a roughly oval scutellum with a pair of circular sensilla located medially to the four primary



scutellar setae (Fig. 2a). Each campaniform sensillum appears externally as a smooth dome in the cuticle surrounded by a raised ring that interrupts the imbricate sculpturing of the scutellum (Fig. 2b). Internally, the cuticle forms a raised ring around an area of reticulate cuticle with an elliptical central depression oriented diagonally to the longitudinal axis of the body (Fig. 2c–d). This ellipse-shaped thinning of the cuticle probably creates a weakness along the long axis of the ellipse and enhances movement along the short axis conferring directional sensitivity similar to that obtained through an elliptically shaped cuticular cap (Moran and Rowley 1975). Across all of the specimens surveyed, the elliptical depression, which is also visible in slide mounts, was found only in *Aphytis* and *Aphelinus* (Aphelininae).

Internally, the scutellum is bordered by several ridges forming a differentiated region directly above the longitudinal flight muscles. Along with the mesoscutello-metanotal muscles (Fig. 3a–e: 114) and randomly distributed fat body (Figs 3e: fb), this space also contains several unidentified structures. In certain dissections examined with SEM (Figs 3c, 4a–b), there appears to be membranous divisions that run through this area defining irregular sections as large as 20  $\mu\text{m}$  in diameter, however these divisions were not apparent in TEM preparations. Just below the cuticle, and between these divisions, there is a single, or sometimes double, layer of elongate epidermal cells (Fig. 4a–i: epd). While tightly packed, these cells appear independent of each other in SEM preparations (Fig. 4a–c) and in TEM preparations appear hollow due to a lack of penetration by the resin. Similar impenetrable epidermal cells also appear in sections of the male antennae prepared by Romani et al. (1999) in their TEM investigation of the male antennae of *A. melinus*. It may be that in the adult wasp the epidermal cells have died leaving a thick waxy cell membrane that is impermeable to resin. These are not likely artifacts of

dried haemolymph which is apparent in the layer of “tissue” surrounding muscle 114 and the sensillar stem (cs) in Fig. 3c. These cells line the entire internal surface of the cuticle, including ventral surfaces (Fig. 4i: epd) and internal apodemes (Fig. 4h: epd), but are absent where the scutellar sensilla attach to the cuticle (Fig. 4g).

*Mesoscutello-metanotal muscles.*—In *A. melinus*, a pair of muscles traverse the length of the scutellum between the longitudinal flight muscles and the dorsal surface of the scutellum (Fig. 3a–b and e), which are synonymous with Kelsey’s (1957) muscle 114 and Vilhelmsen’s (2000) mesoscutello-metanotal muscle. The muscles attach to the anterior portion of the scutellum just posterior to the scutoscutellar ridge (Fig. 3a–c: 114 and ssr). From this point of origin they narrow and are slightly angled medially to a posterior insertion to the anterior edge of the metanotum above the margin of the second phragma (2ph) to the anterior edge of the metanotum (Fig. 3a–b: 114). In cross section, the longitudinal flight muscles have clearly defined axon bundles interspersed with mitochondria (Fig. 3d–e: 112), whereas the mesoscutello-metanotal muscle has mitochondria restricted to the periphery. Consequently axon bundles are not as easily distinguished (Fig. 3d–e). These muscles may affect longitudinal tension of the scutellar disc and possibly deformation of shape in small soft-bodied chalcidoids.

*Ultrastructure of the sensillar cuticular cap.*—In *A. melinus*, there are several distinct features of the cuticular portion of the scutellar sensilla evident through electron microscopy. In cross sections there is a thin outer layer of solid cuticle. This layer (Fig. 5a–e: L1) sits external to a thicker layer of mesh-like cuticle (Fig. 5a–f: L2). These two layers of the cap are consistent with the cuticular structure found in campaniform sensilla observed in other studies where 2 or 3 layer-caps are reported (McIver 1985) and it is nearly

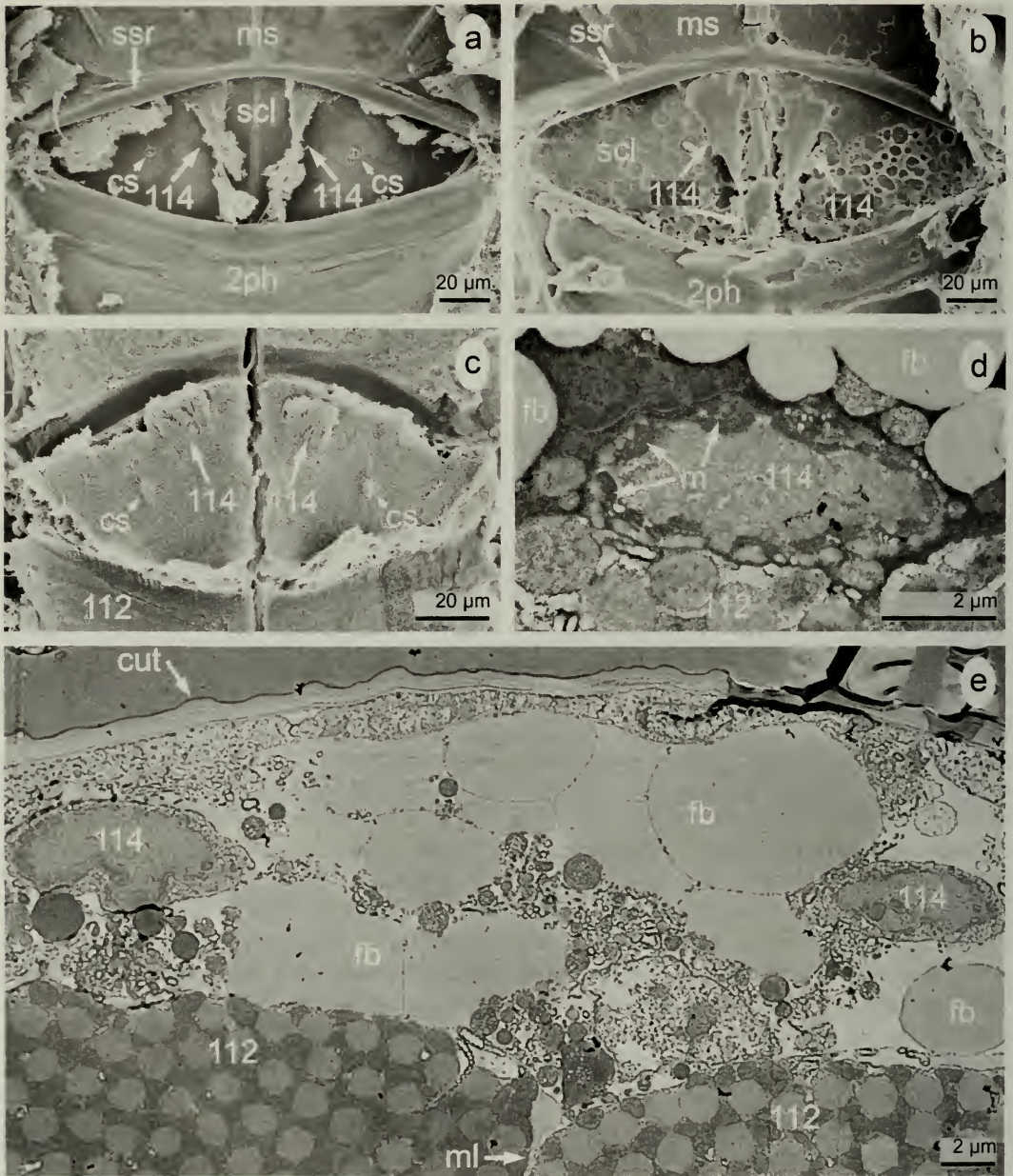


Fig. 3. Mesoscutello-metanotal muscles in *Aphytis melinus*. a: underside of scutellum with most tissue removed leaving mesoscutello-metanotal muscles (114), b: same view in (a) with slightly different results from the chemical drying process, c: dorsal tissue found just beneath scutellum, d: cross section through mesoscutello-metanotal muscle, e: cross section through dorsal portion of scutellum.

identical to the structure observed by Bromley et al. (1980) in aphid antennae. The bilayered cap is encircled by a flange that protrudes internally. This flange was observed by McIver and Siemicki (1975) in the mosquito, and in the cockroach by

Moran and Rowley (1975), who called the structure a cuticular collar. Moran and Rowley also suggested that it provides structural support and rigidity for the cap of the sensilla and enables the cap to move as a unit in response to cuticular deforma-



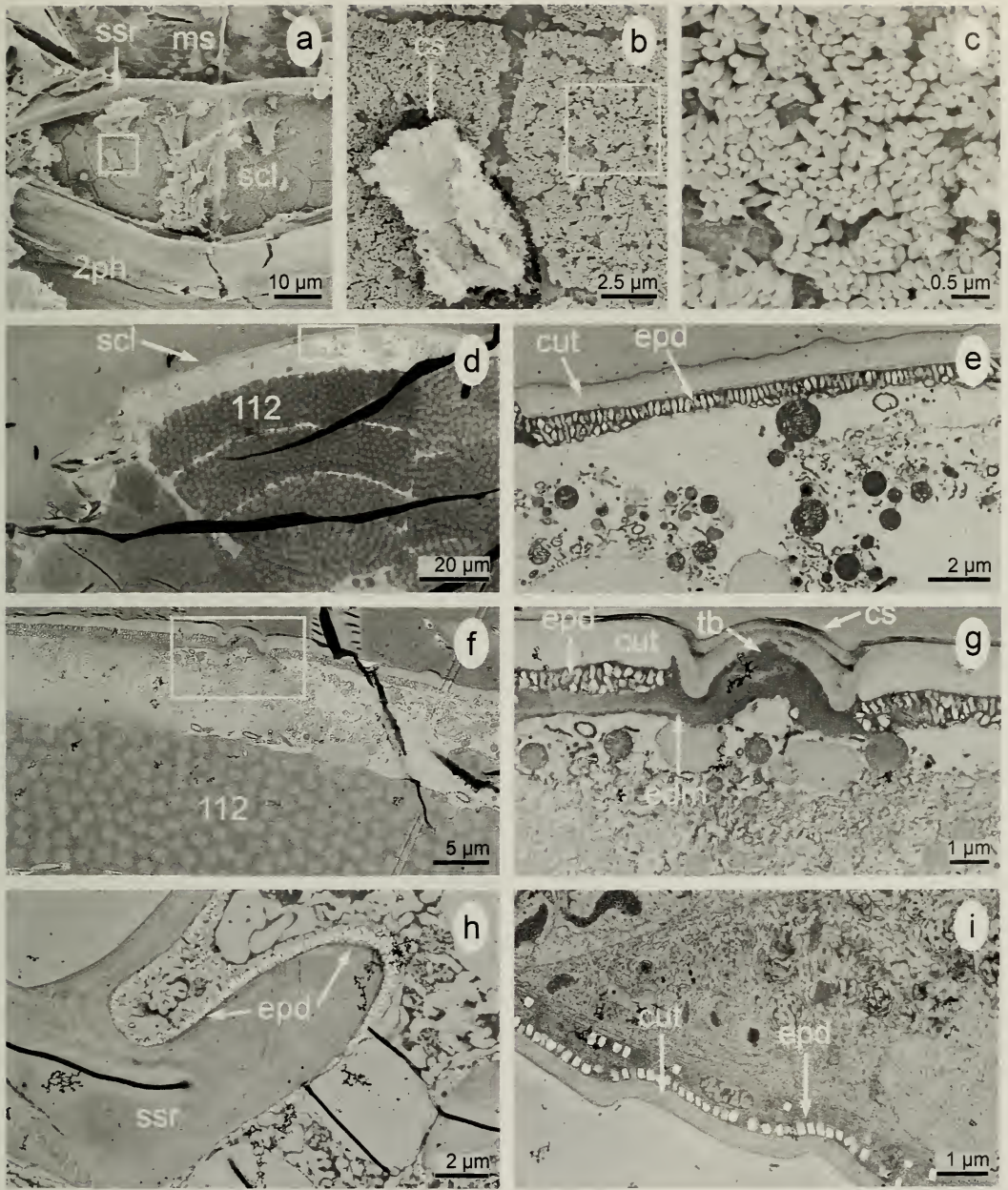


Fig. 4. Epidermal cells in *Aphytis melinus*. a: underside of scutellum with most tissue removed leaving epidermal cells, b: underside of scutellum with campaniform sensillum tissue and epidermal cells attached, c: underside of sensilla with epidermal cells attached, d–e: cross section through scutellum, f–g: cross section through scutellum with a campaniform sensillum, h: cross section through notal ridge, i: cross section through ventral portion of mesosoma. White boxes indicate area magnified in following figure.

tion. Just dorsal to the flange, layer 1 is attached to the cuticle by a ring of dark staining cuticle (Fig. 5a–c) that McIver and Siemicki (1975) called a hinge. This design-

ation seems appropriate since it is at this junction point, at the base of the flange where the cap and cuticle meet, that the cuticle would presumably bend.

*Tubular body and electron dense mass.*—One of the most distinctive features of a campaniform sensilla is the tubular body at the distal end of the nerve cell (McIver 1985), which is a bundle of microtubules set in an electron dense material that functions as the site of transduction (Thurm 1964). In *A. melinus*, the tubular body is a striated cap that inserts into layer two of the cuticle, almost extending to layer one (Fig. 5d: tb). The tubular body consists of microtubules perpendicularly oriented to the surface of the cuticle and set in an electron-dense material. An electron-lucent area located at the proximal end separates the tubular body from the electron-dense mass beneath (Fig. 5c: ela and edm). In some preparations, the tubular body appears to have a slightly indented tip in the very center of its distal end (Fig. 5e). The proximal end of the cap-like tubular body is nested in an electron-dense mass (Fig. 5c: edm). This dense material surrounds the tubular body and is directly adjacent to the modified portions of the cuticular cap, completely filling the sunken areas below layer two and surrounding the flange (Fig. 5b–c). It also extends beyond the campaniform sensillum, particularly in the lateral direction, to form a large matt beneath the cuticle (Fig. 6a–d). The electron-dense mass appears to consist of microtubules or lamella similar to the tormogen cell associated with campaniform sensilla found on mosquito palps (McIver 1985), but appears to lack other cellular structures indicative of a tormogen cell such as a membrane bound nucleus (Thurm and Küppers 1980). No other dendritic cells were identified in association with the campaniform sensilla.

*Distribution of sensilla across Chalcidoidea.*—The paired scutellar sensilla are found in most families of Chalcidoidea and in exemplars of four outgroup families (Ceraphronidae, Mymarommatidae [single sensillum], Scelionidae and Diapriidae). In prepared slides, the sensilla appear as pale spots or thin areas in the cuticle and are

readily identified in smaller taxa such as *Aphytis* (Rosen and DeBach 1979). In SEM preparations, they appear externally as differentiated areas of the cuticle that break the cuticular pattern and typically are ringed by raised or depressed cuticle (Figs 7a–h, 8a–h). The location and shape of the sensilla on the scutellum are highly variable across Chalcidoidea, but there is consistency within taxonomic groups at the family, tribe, genus and species levels (Romero and Heraty, in prep.). The location of the sensilla varies from medially abutting in some Aphelinidae, Encyrtidae and Mymaridae, to a lateral location within 5  $\mu\text{m}$  of the edge of the scutellum in some Pteromalidae and Eulophidae. Sensillar location also varies along the longitudinal axis from an anterior location contiguous with the scutoscutellar suture (sss) in some Aphelinidae and Mymaridae to a posterior location within a few microns of the posterior margin in some Mymaridae. The sensilla are always found anterior to the frenal line when a frenum is present. The most common location is generally central and just medial of the anterior and posterior scutellar setae when present (Fig. 2a). The shape of the sensilla range from circular (Fig. 7a), to longitudinally oblong (Fig. 7d), to transversely oblong (Fig. 7c), with circular being the predominant shape. For the subset of representative specimens measured, the diameter of the sensilla ranges from 1.81  $\mu\text{m}$  in *Orasema* sp. (Eucharitidae) to 5.79  $\mu\text{m}$  in *Microterys nietneri* (Motschulsky) (Encyrtidae) (Table 2).

Comparisons of sensillar diameter and scutellar length, width and area revealed that scutellar size accounts for very little variation in sensillar diameter (Fig. 9a–c). R-squared values were low with the highest value at 0.081 (Fig. 9a). Comparisons of scutellar length and sensillar diameter had an R-squared value of 0.024 (Fig. 9b), and scutellar area and sensillar diameter had an R-squared value of 0.057 (Fig. 9c). The low R-squared values for the regression lines indicate that the variation in the size



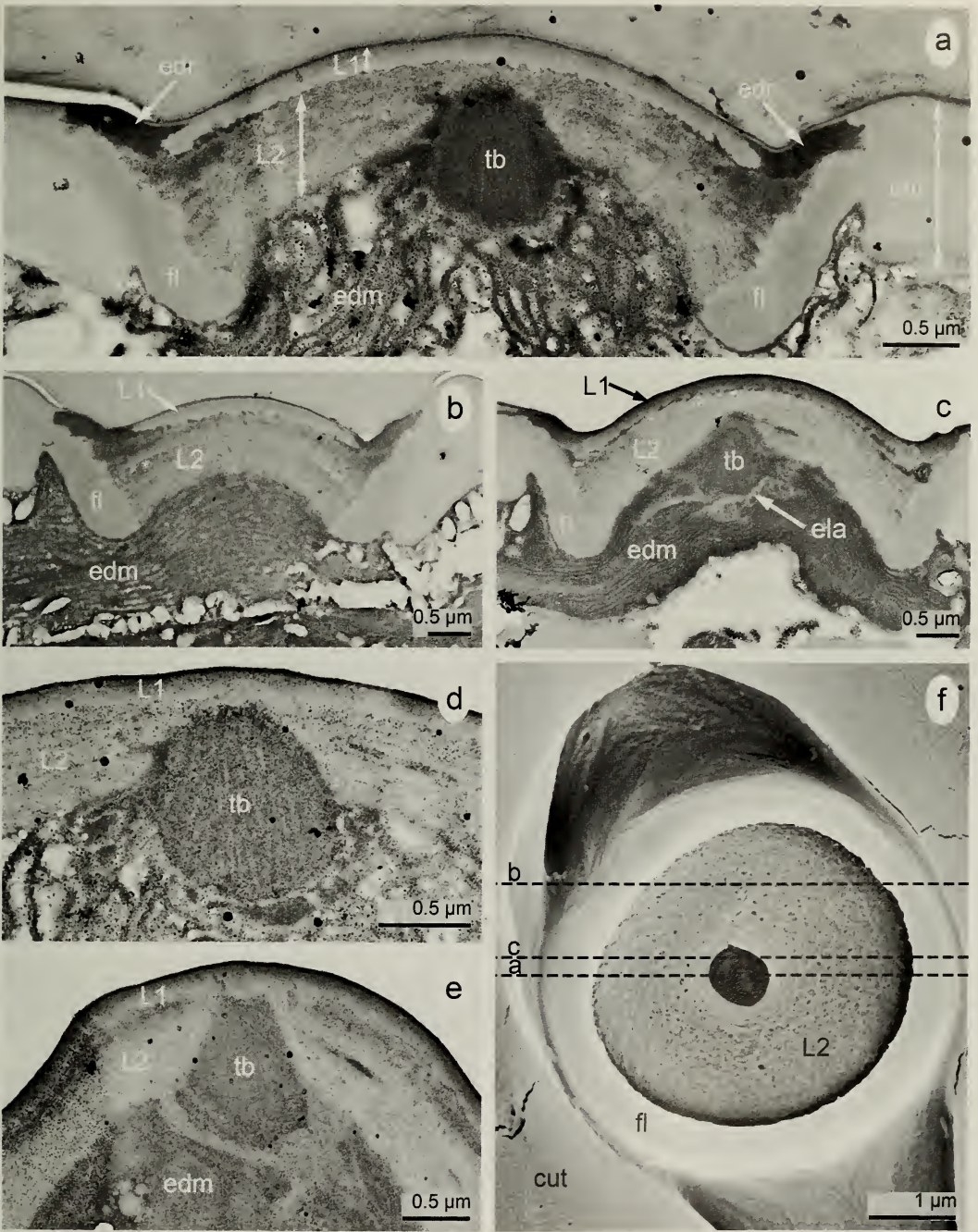


Fig. 5. *Aphytis melinus*, multiple specimens. a–c: cross sections through portions of campaniform sensilla shown in (f), d: cross section through the tubular body, e: cross section through the tubular body showing divot at tip, f: the underside of a campaniform sensillum with tissue removed, dashed lines indicate the general location of cross sections in (a), (b) and (c).



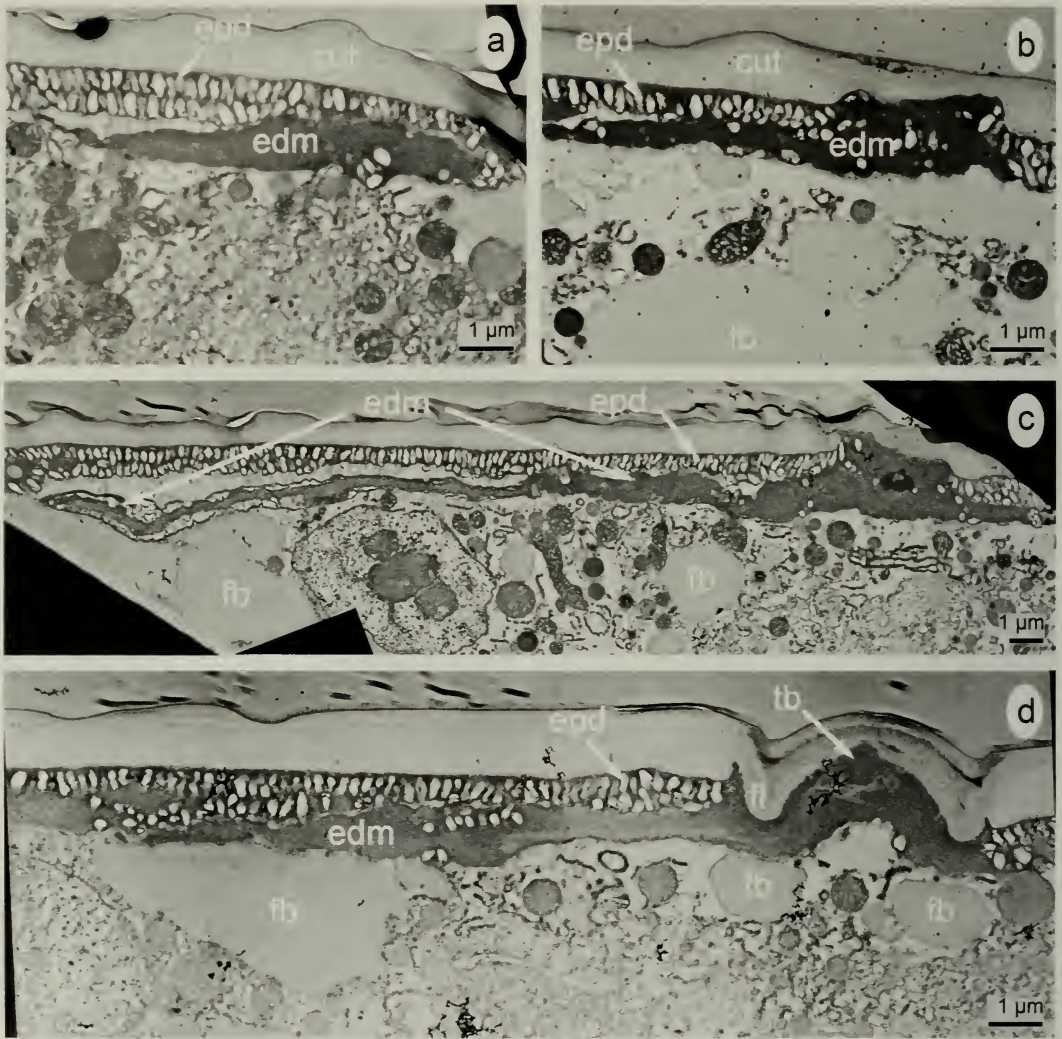


Fig. 6. Electron-dense mass associated with the campaniform sensillum to the left of the midline in *Aphytis melinus*. a: cross section anterior to campaniform sensillum, b–c: cross sections just anterior to the main structure of the campaniform sensillum, d: cross section through the center of the campaniform sensillum.

of the scutellum accounts for less than 9% of the variation in the diameter of the sensilla. While there does not appear to be a relationship between size of scutellum and size of sensilla, the sensilla are absent or undetectable in chalcidoid families with the largest species, which have a scutellar size far beyond that indicated in Fig. 9 (i.e. Chalcididae and Perilampidae, >2 mm) (Romero and Heraty, in prep.). Thus our correlations are based only on taxa that are normally small in size, not those taxa that are large. A similar situation occurs in the

outgroups, and we failed to find evidence of the sensilla in the majority of species which are usually larger or more heavily sclerotized. Strong correlations do appear within species (i.e. *A. melinus*, Fig. 9), but these were not correlated across subfamily or family groups.

## DISCUSSION

This is the first study to examine the morphology of scutellar sensilla of Chalcidoidea. The presence of sensilla has been noted sporadically in the literature, and



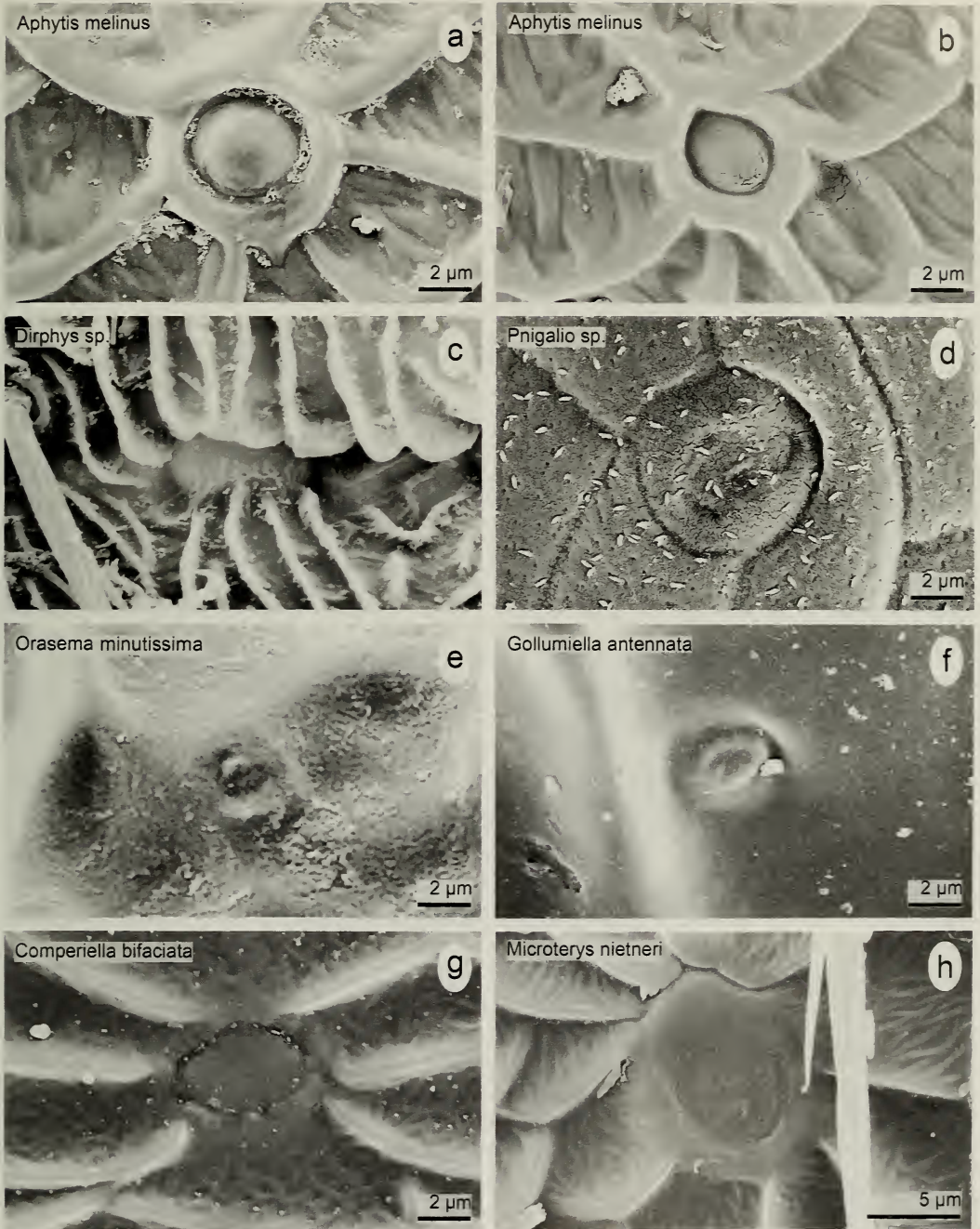


Fig. 7. Variations in external appearance of scutellar sensilla. a: *Aphytis melinus* ♀ (Aphelinidae), b: *A. melinus* ♂, c: *Dirphys* sp. (Aphelinidae), d: *Pnigalio* sp. (Eulophidae), e: *Orasema minutissima* (Eucharitidae), f: *Gollumiella antennata* (Eucharitidae), g: *Comperiella bifaciata* Howard (Encyrtidae), h: *Microterys nietneri* (Encyrtidae).

often included in illustrations without comment in the text. The most attention this feature has received is in *Encarsia* (Aphelinidae), where relative placement

on the scutellum has been used in several keys to discriminate both individual species and species groups (Hayat 1998; Hernández-Suárez et al. 2003; Schauff et

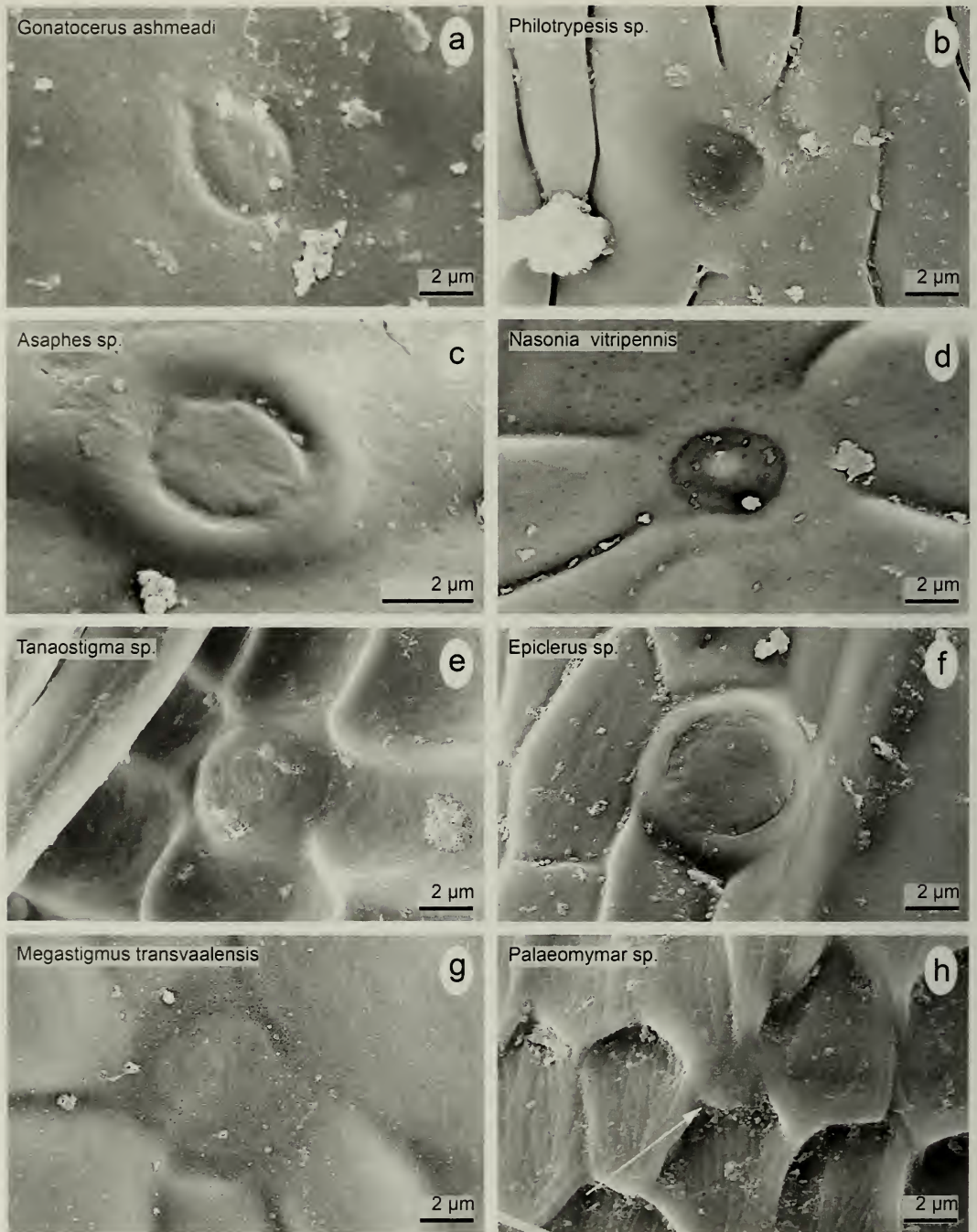


Fig. 8. Variations in external appearance of scutellar sensilla. a: *Gonatocerus ashmeadi* (Mymaridae), b: *Philotrypesis* sp. (Pteromalidae), c: *Asaphes* sp. (Pteromalidae), d: *Nasonia vitripennis* (Pteromalidae), e: *Tanaostigma* sp. (Tanaostigmatidae), f: *Epiclerus* sp. (Tetracampidae), g: *Megastigma transvaalensis* (Torymidae), h: *Mymaromma anomalum* (Mymarommatidae). Arrow indicates location of sensillum.



al.1996). The sensilla have been referred to as scolopophorous sensilla (Gordh 1975b), and more recently as scutellar sensilla (Babcock et al. 2001; Hayat 1998; Heraty and Polaszek 2000), but most often as placoid sensilla (Annecke and Doult 1961; Hayat 1998; Kim 2003; Rosen and DeBach 1979; Schauf 1984). Both scolopophorous and placoid refer to specific types of sensilla described by Snodgrass (1935). Scolopophorus sensilla, also known as chordotonal organs, are composed of bundles of sensory cells that attach to a specific point on the cuticle in order to detect vibration. Placoid sensilla are composed of multiple sense cells and function as olfactory sensilla, often with numerous pores through the cuticle. The sensilla found on the chalcidoid scutellum do not possess pores, and are innervated by a single nerve cell as indicated by the lone tubular body (Fig. 6a). They are best defined as campaniform sensilla, which function as mechanoreceptors (Pringle 1938a).

Scutellar sensilla are found in chalcidoid families with small-bodied species, but generally not in families with the largest-bodied species. There does not seem to be an absolute size at which the sensilla are consistently absent, but rather a trend where chalcidoid families with consistently small members such as Aphelinidae, Encyrtidae and Trichogrammatidae possess the sensilla, those with consistently large members such as Chalcididae and Perilampidae do not, and those with intermediate-sized members such as Eulophidae and Torymidae have members with and without the sensilla (Romero and Heraty, in prep.). Presence does not appear to be necessarily correlated with degree of body sclerotization, as sensilla are retained in both larger members of soft-bodied Eulophidae and small well-sclerotized members of some Pteromalidae and Eucharitidae. Scutellar sensilla were found in the outgroup families Ceraphronidae, Diapriidae, Scelionidae and Mymarommatidae. In these families the sensilla are only

present in smaller species as is the case within Chalcidoidea. This would indicate that presence of scutellar sensilla is plesiomorphic for Chalcidoidea, and their subsequent loss derived.

In chalcidoids that possess scutellar sensilla, the sensilla vary in size, shape and location. The shape varies from circular to oval, with both shapes commonly observed in campaniform sensilla recorded from other studies (McIver 1985). The size of the sensilla in the subset of taxa measured ranges from 1.81  $\mu\text{m}$  to 5.79  $\mu\text{m}$ . While this is over a 3 fold difference, campaniform sensilla have been recorded as small as 1  $\mu\text{m}$  (Hawke et al. 1973) and as large as 30  $\mu\text{m}$  in other insects (Hustert et al. 1981). Chalcidoid scutellar sensilla generally fall in the 1.5–10  $\mu\text{m}$  range as reported for most campaniform sensilla (Amornsak et al. 1998; Blaney and Chapman 1969; Chevalier 1969; McIver 1985; Moran and Rowley 1975; Schmidt and Smith 1985, 1987).

Variation in size of the sensilla does not seem to be closely tied to the size of the scutellum (Fig. 9a–c). The low R-squared values ( $\leq 0.081$ ) indicate that size of the scutellum is a poor predictor of the size of the sensilla. In several specimens such as *Gollumiella antennata* (Gahan) (Eucharitidae) and *Microterys nietneri*, there is as much as a 0.87  $\mu\text{m}$  difference between the left and right sensilla on the same specimen. In species represented by multiple specimens, such as *A. melinus*, there appears to be a relation between size of sensilla and size of scutellum with larger specimens possessing larger sensilla (Fig. 9a–c: *A. melinus*). This intraspecific trend between sensillar size and specimen size was also observed by Schmidt and Smith (1985) in their examination of wing sensilla in 18 specimens of *Trichogramma minutum* Riley (Trichogrammatidae).

The shape of the campaniform sensilla has been shown to confer directional sensitivity (Pringle 1961). Circular sensilla do not have directional sensitivity whereas

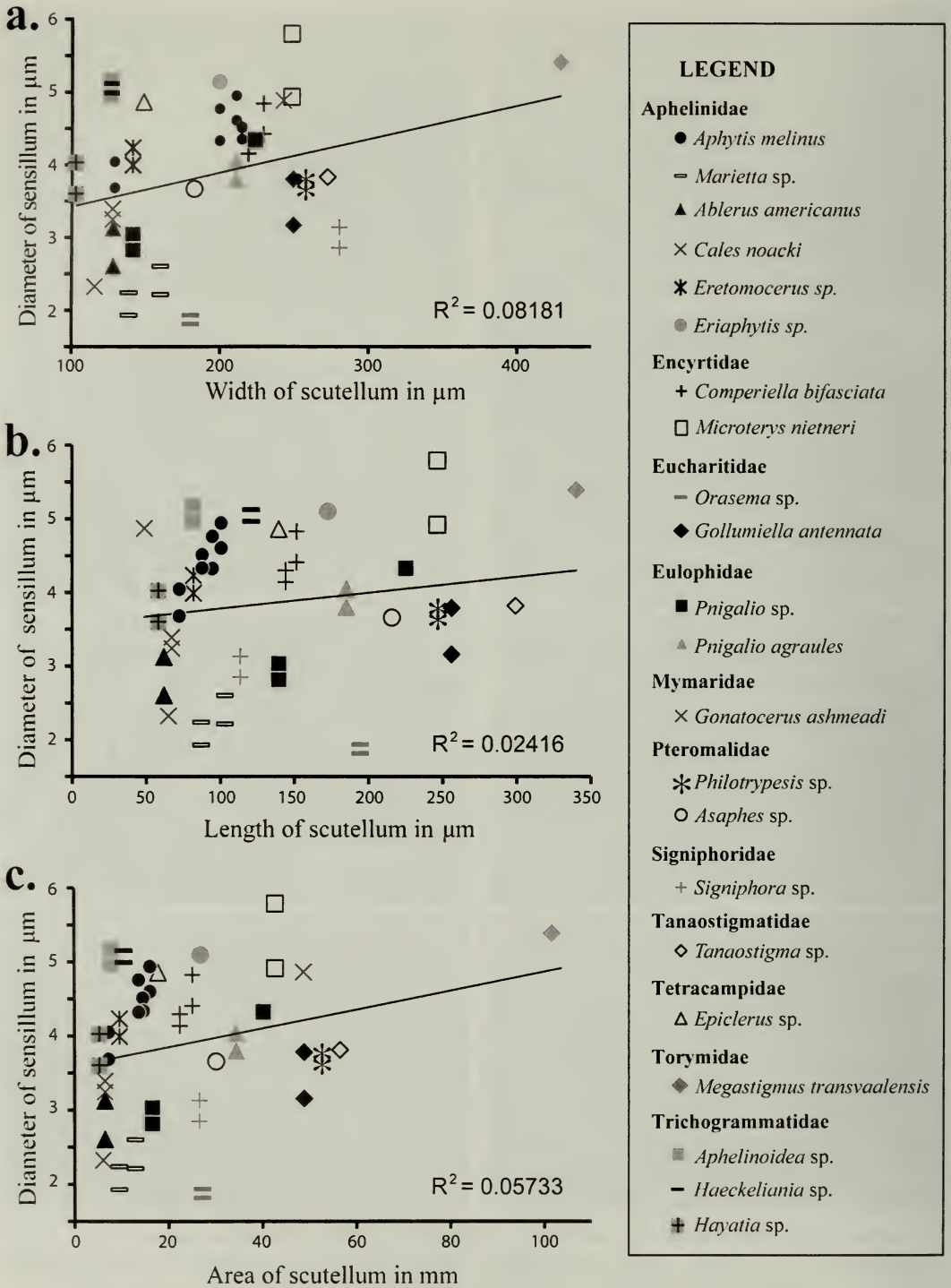


Fig. 9. Diameter of scutellar sensilla and size of scutellum for various chalcidoids. a: Scutellum width and sensillum diameter. b: Scutellum length and sensillum diameter. c: Scutellum area and sensillum diameter.



oval sensilla have more sensitivity to cuticular strain along the major axis (Moran and Rowley 1975). In chalcidoids, the scutellar sensilla can be circular or oval, with oval sensilla found oriented transversely in some Aphelinidae and longitudinally in some Eulophidae and Pteromalidae (Romero and Heraty, in prep.). The variability in the shape as well as in the location of the sensilla on the scutellum is most likely related to the variable morphology of the scutellum and the mesonotum as a whole. Different combinations of morphological features such as thickness and shape of the scutellum, placement of internal ridges, and relation of the scutellum to other sclerites could be associated with diverse complimentary positions of mechanoreceptors such as the scutellar sensilla. The consistent presence of the sensilla across entire families seems to indicate that there is strong selective pressure to maintain the sensilla. The symmetric variation in shape and position of the sensilla and consistency within phylogenetic lineages (Romero and Heraty, in prep.), further support the hypothesis that variation in the sensilla is tied to optimal functionality.

Scutellar sensilla are notably absent in taxa with larger members. One explanation for the exclusive presence of the sensilla in smaller chalcidoids is that these smaller insects employ unique flight techniques not often utilized by larger insects. In his paper on flight and lift production, Weis-Fogh (1973) observed a novel wing motion mechanism in small (1–2 mm) *Encarsia formosa* Gahan (Aphelinidae), later termed clap-and-fling. This mechanism involves the right and left wing meeting, or clapping, at the end of the up-stroke and beginning of the down-stroke and has been observed in other small insects with low Reynolds numbers such as the greenhouse whitefly (Weis-Fogh 1975) and thrips (Ellington 1984). Miller and Peskin (2005) have shown that clap-and-fling provides less of a lift enhancing effect for insects

with intermediate Reynolds numbers, such as the fruit fly, *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) ( $Re=64$ ). While larger insects are known to use clap-and-fling when tethered (Vogel 1966; Götz 1987; Zanker 1990), when carrying large loads (Marden 1987), and when performing certain steering maneuvers (Cooter and Baker 1977; Ellington 1984), it seems very small insects are obliged to utilize clap-and-fling on a regular basis (Lehmann et al. 2005; Miller and Peskin 2005). The scutellum is uniquely placed between the junction of the wing base and the lateral anterior connection of the posterior attachment of the longitudinal flight muscles (2<sup>nd</sup> phragma) and thus could play an important role in monitoring flight activity. As mechanoreceptors, the campaniform sensilla are likely measuring changes in the torsion and tension of the scutellar cuticle. It specifically relates to a small insect flight specialization such as clap-and-fling, and consequently scutellar sensilla are consistently retained in the smallest of chalcidoids and other Hymenoptera.

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